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14. ABSTRACT Arena 60 Discrete Photometric Analyzer System and ancillary instrumentation were acquired to increase our analytical capability, increase laboratory efficiency, and improve overall research effectiveness within the three primary research focus areas namely: (1) mitochondrial functional analysis of oxidative energy production associated with nutrient efficiencies, growth and development in different life stages of rainbow trout, (2) Aqueous metal-ion complexation utilizing solid-supported water soluble ligands, and (3) Enzymatic glycan treatment and characterization of glycan profiles in pre- and post-treatment. Our long-term goal is to increase our research					
15. SUBJECT TERMS spectrophotometric analyzer, Arena 60, Mitochondria, metal-ion complexation, rainbow trout					
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a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			Jonathan Eya
					19b. TELEPHONE NUMBER 304-766-4260



## Report Title

Final Report: An Automated Analyzer System to Strengthen Teaching and Research Infrastructure at West Virginia State University

### ABSTRACT

Arena 60 Discrete Photometric Analyzer System and ancillary instrumentation were acquired to increase our analytical capability, increase laboratory efficiency, and improve overall research effectiveness within the three primary research focus areas namely: (1) mitochondrial functional analysis of oxidative energy production associated with nutrient efficiencies, growth and development in different life stages of rainbow trout, (2) Aqueous metal-ion complexation utilizing solid-supported water soluble ligands, and (3) Enzymatic glycan treatment and characterization of glycan profiles in pre- and post-treatment. Our long-term goal is to increase our research capacity that will facilitate educating and training students from backgrounds that are underrepresented in STEM fields via the utilization of the newly acquired instrumentation.

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**Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:**

**(a) Papers published in peer-reviewed journals (N/A for none)**

Received

Paper

**TOTAL:**

**Number of Papers published in peer-reviewed journals:**

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**(b) Papers published in non-peer-reviewed journals (N/A for none)**

Received

Paper

**TOTAL:**

**Number of Papers published in non peer-reviewed journals:**

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### (c) Presentations

Yossa, R., O.O. Okubajo, D.A. Perera, I. Pushpalingam, J.C. Eya. 2014. Interactive effects of dietary protein sources, dietary lipid levels and water temperatures on growth, feed utilization, enzyme activities and gene expressions in rainbow trout. Aquaculture America 2014, International Conference and exposition, February 9-12, 2014, Seattle, Washington, USA. Abstract ID# 580. (Oral)

Number of Presentations: 1.00

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**Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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**Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

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**(d) Manuscripts**

Received      Paper

**TOTAL:**

Number of Manuscripts:

Books

Received      Book

TOTAL:

Received      Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

<u>NAME</u>	<u>PERCENT_SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT_SUPPORTED</u>
FTE Equivalent:	
Total Number:	

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### Names of Faculty Supported

NAME

PERCENT SUPPORTED

**FTE Equivalent:**

**Total Number:**

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### Names of Under Graduate students supported

NAME

PERCENT SUPPORTED

**FTE Equivalent:**

**Total Number:**

### Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ..... 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: ..... 0.00

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### Names of Personnel receiving masters degrees

NAME

**Total Number:**

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### Names of personnel receiving PHDs

NAME

**Total Number:**

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### Names of other research staff

NAME

PERCENT SUPPORTED

**FTE Equivalent:**

**Total Number:**

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**Sub Contractors (DD882)**

## **Inventions (DD882)**

### **Scientific Progress**

#### **Principal Accomplishments:**

- a. One Postdoctoral fellow was trained using the automated Arena 60 Discrete Photometric Analyzer and associated equipment, and was able to develop and validate protocols for the analyses of mitochondrial complexes enzymatic activities.
- b. One graduate student obtained Master of Science in Biotechnology and utilized the instrumentations in the generation of data for his Master of Science thesis.
- c. Currently, another graduate student is using the instrumentations for analyses of samples for his Master Science thesis while three undergraduate students are using the equipment for the directed student research to fulfill their requirements for honors program in the Department of Biology.
- d. Future research focus of the PD that involves high throughput enzyme kinetics and biochemical analyses requiring spectrophotometric analyses will be performed with this instrumentation. Other instruments purchased with funds from this project will be used in future research undertaken by the PD .

#### **Recently Published Papers on Mitochondria Energetics:**

Eya, J.C., Ukwuaba, V.O., Yossa, R. and Gannam , A.L. 2015. Interactive Effects of Dietary Lipid and Phenotypic Feed Efficiency on the Expression of Nuclear and Mitochondrial Genes Involved in the Mitochondrial Electron Transport Chain in Rainbow Trout. *International Journal of Molecular Sciences* 16: 7682-7706; doi:10.3390/ijms16047682.

Eya, J.C., Ukwuaba, V.O., Yossa, R., Ashame, M.F., Pomeroy, C.F., Gannam, A.L. 2015. Growth performance and mitochondrial function in juvenile rainbow trout (*Oncorhynchus mykiss*) fed graded dietary lipid levels. *Annals of Aquaculture and Research* 2(1): 1006-1018.

Eya, J.C., Nouaga, R.Y., Ashame, M.F., Pomeroy, C.F., Gannam, A.L. 2014. Effects of dietary lipid levels on mitochondrial gene expression in low and high-feed efficient families of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Biology* 84: 1708-1720.

#### **Presentation Mitochondria Energetics:**

Yossa, R., O.O. Okubajo, D.A. Perera, I. Pushpalingam, J.C. Eya. 2014. Interactive effects of dietary protein sources, dietary lipid levels and water temperatures on growth, feed utilization, enzyme activities and gene expressions in rainbow trout. *Aquaculture America 2014, International Conference and exposition, February 9-12, 2014, Seattle, Washington, USA. Abstract ID# 580. (Oral)*

#### **Summary of the research Project:**

The PD is involved in research activities that span nutrition, biochemistry, physiology, molecular biology and genomics and has utilized these instruments sample preparations and analyses. PD and his research group have studied and continue to study the interface between dietary manipulations, temperature and nuclear- and mitochondrial-encoded genes involved in oxidative metabolism; and determine the potential role of nutrients and non-dietary factors (temperature) in the molecular modulation of protein and gene expression levels that influence nutrient retention efficiency, growth and development in fishes at different live history stages. These acquired instrumentation together with existing equipment have contributed to successful completion of projects funded by other federal government agencies and the results from various studies are contained in the publications listed above.

### **Technology Transfer**

## FINAL TECHNICAL REPORT

### *An Automated Analyzer System to strengthen teaching and research infrastructure at West Virginia State University*

**Equipment Purchased:** The following instrumentations were purchased from different vendors:

1. Thermo Fisher Scientific Arena 60 Discrete Photometric Analyzer (\$125,000); Vendor: Thermo Fisher Scientific, 46360 Fremont Blvd, Fremont, CA 94538, 732-371-3870, [www.thermo.com](http://www.thermo.com), Catalog or Part number: 984138.
2. Tissue Lyser II with tungsten carbide beads, 96 well 3 mm bead dispenser, and adapter sets (\$12,254.40); Vendor: QIAGEN Inc., 27220 Turnberry Lane, Suite 200, Valencia, CA 91355-1005, 1-800-718-2056, [www.qiagen.com](http://www.qiagen.com), Catalog or Part number: 85300, 69997, 69982, 69984, 69973.
3. Centrifuge, multipurpose, Eppendorf, Model 5810R with 4 x 500 mL Rotor, 4 x 13 mm O.D. Adapters and 4 x 16 mm O.D. Adapters (Clinical Package) (\$13,508.25); Vendor: Fisher Scientific Company LLC, 4500 Turnberry Drive, Hanover Park, IL 60133, 1-800-766-7000, [www.fishersci.com](http://www.fishersci.com), Catalog or Part number: 05-413-326.

**Technical Summary of the Project:** The specific objectives of this grant request were: 1) to secure funds for the purchase of an automated chemistry analyzer system with the ancillary instrumentations such as tissue Lyser II and refrigerated multipurpose centrifuge, which are necessary for the successful continuance and expansion of current research programs of the project director (PD) and collaborating scientists, and 2) to increase the participation of underrepresented students in STEM disciplines such as chemistry and biology through familiarity with the use of an advanced equipment such as the requested automated analyzer system.

The research projects that benefited from the purchase of the automated Arena 60 Discrete Photometric Analyzer are: 1) Mitochondrial Oxidative Bioenergetics, 2) Aqueous Metal-Ion Complexation Utilizing Solid-Supported Water-Soluble Ligands, and 3) Enzymatic Glycan Treatment and Characterization.

#### *Project 1: Mitochondrial Oxidative Bioenergetics*

##### *Principal Accomplishments:*

- a. One Postdoctoral fellow was trained using the automated Arena 60 Discrete Photometric Analyzer and associated equipment, and was able to develop and validate protocols for the analyses of mitochondrial complexes enzymatic activities.
- b. One graduate student obtained Master of Science in Biotechnology and utilized the instrumentations in the generation of data for his Master of Science thesis.



- c. Currently, another graduate student is using the instrumentations for analyses of samples for his Master Science thesis while three undergraduate students are using the equipment for the directed student research to fulfil her requirements for honors program in the Department of Biology.
- d. Future research focus of the PD that involves high throughput enzyme kinetics and biochemical analyses requiring spectrophotometric analyses will be performed with this instrumentation. Other instruments purchased with funds from this project will be used in future research undertaken by the PD.

*Recently Published Papers on Mitochondria Energetics:*

- Eya, J.C., Ukwuaba, V.O., Yossa, R. and Gannam, A.L. 2015. Interactive Effects of Dietary Lipid and Phenotypic Feed Efficiency on the Expression of Nuclear and Mitochondrial Genes Involved in the Mitochondrial Electron Transport Chain in Rainbow Trout. *International Journal of Molecular Sciences* 16: 7682-7706; doi:10.3390/ijms16047682.
- Eya, J.C., Ukwuaba, V.O., Yossa, R., Ashame, M.F., Pomeroy, C.F., Gannam, A.L. 2015. Growth performance and mitochondrial function in juvenile rainbow trout (*Oncorhynchus mykiss*) fed graded dietary lipid levels. *Annals of Aquaculture and Research* 2(1): 1006-1018.
- Eya, J.C., Nouaga, R.Y., Ashame, M.F., Pomeroy, C.F., Gannam, A.L. 2014. Effects of dietary lipid levels on mitochondrial gene expression in low and high-feed efficient families of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Biology* 84: 1708-1720.

*Presentation Mitochondria Energetics:*

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*Summary of the research Project:*

Nutrigenomics (Mitochondrial Energetics): The PD is involved in research activities that span nutrition, biochemistry, physiology, molecular biology and genomics and has utilized these instruments sample preparations and analyses. PD and his research group have studied and continue to study the interface between dietary manipulations, temperature and nuclear- and mitochondrial-encoded genes involved in oxidative metabolism; and determine the potential role of nutrients and non-dietary factors (temperature) in the molecular modulation of protein and gene expression levels that influence nutrient retention efficiency, growth and development in fishes at different live history stages. These acquired instrumentation together with existing equipment have contributed to successful completion of projects funded by other federal

government agencies and the results from various studies are contained in the publications listed above.

The Arena 60 Discrete Photometric Analyzer was used in high throughput enzyme kinetic analyses and protein determination.

The tissue Lyser II and refrigerated multipurpose centrifuge were used in sample preparation for biochemical and gene expression analyses.

**Aqueous Metal-ion Complexation:** This work has focused on the extraction of Fe(II), and Zn(II), ions from aqueous solution utilizing ligands mounted on an insoluble solid support. Four extractors, identified as Ext. 1, Ext. 3, Ext.5, and 3-aminopropyl-functionalized silica gel (APFSG) were utilized. Batch extractions were performed followed by filtration. The Fe(II) and Zn(II) ion concentrations in the filtrates were determined by redox and EDTA titrations respectively. Extractions of over 90% of the metal ions were observed with APFSG. The next phase of this project shall utilize the Arena 60 Discrete Photometric Analyzer to determine the extent of extraction of the anions, e.g.,  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$ .

**Enzymatic Glycan treatment and characterization:** Currently, there is development and validation of protocols for the detection of glycosylation changes and the characterization of glycans, and Arena 60 Discrete Photometric Analyzer will be optimized and utilized to characterize N-linked glycans, in an automated workflow solution. The Arena 60 will be for analyses of the synthesized mutant proteins for rapid analytical comparisons.